Allowed claims

1. A method of producing a plurality of overlapping double stranded (ds) RNA fragments of a size in the range of about 15-30 nucleotides, comprising:

(a) digesting a preparation of large double-stranded RNA in a reaction mixture containing a divalent transition metal cation and a prokaryotic RNaseIII wherein the ratio of enzyme to substrate (w/w) is greater than or equal to about 0.25:1; and

(b) producing the plurality of overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides.

2. A method according to claim 1, wherein the plurality of overlapping fragments is the product of complete digestion of the preparation of large double-stranded RNA.

3-4 (cancelled)

5. A method according to claim 1, wherein the transition metal cation is manganese.

6. A method according to claim 5, wherein the reaction mixture contains manganese ions at a concentration in the range of about 5-10 mM.

7. A method according to claim 5, wherein the reaction mixture contains manganese ions at a concentration in the range of about 10-20 mM.

8. A method according to claim 1, wherein the transition metal is selected from nickel, cobalt and cadmium.

9. A method according to claim 2, wherein the complete digestion is achieved in less than 6 hours.

10. A method according to claim 2, wherein the complete digestion is achieved in less than 2 hours.

11. (cancelled)

12. A method of silencing expression of a target gene, comprising: introducing into a host cell, a plurality of fragments made according to claim 1, wherein the nucleotide sequence for each fragment has a sequence that is complementary to the target gene.

13. A purified set of double-stranded RNA fragments, comprising a plurality of overlapping fragments of a size in the range of about 15-30 nucleotides, the fragments in the set collectively representing a substantial portion of a sequence of one or more large double-stranded RNAs from which the fragments are derived by in vitro cleavage with a purified enzyme, one strand of each of the large double-stranded RNA having a sequence complementary to part or all of a target RNA.

14. A set of fragments according to claim 13, wherein the substantial portion is greater than about 50% of the sequence of the large double-stranded RNA.

15. A set of fragments according to claim 13, wherein the substantial portion is greater than about 65% of the sequence of the large double-stranded RNA.

16. A set of fragments according to claim 13, wherein more than about 30% of the RNA fragments have a fragment size of about 18-25 base pairs.

17. A set of fragments according to claim 13, wherein at least one fragment and as many as 100% of fragments in the set are capable of causing cleaving the target RNA in a cell.

18. A set of fragments according to claim 17, wherein at least about 50% of the fragments are capable of causing cleavage of the RNA.

19. A set of fragments according to claim 17, wherein at least about 75% of the fragments are capable of causing cleavage of the mRNA.

20. A set of fragments according to claim 13, capable of RNA silencing in vivo when introduced into a eukaryotic cell.

21. A purified set of double-stranded RNA fragments according to claim 13, wherein the fragments bind specifically to mRNA to initiate cleavage of the mRNA.